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The Fate and Transport of *Cryptosporidium parvum* Oocysts in the Soil

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1. Introduction

Cryptosporidium parvum (*C. parvum*) is a protozoan pathogen that is commonly present in surface water used for recreation, treated and untreated sewage and even drinking water. The zoonotic protozoan parasite *C. parvum* poses a significant risk to public health and has become a global concern to the water resource management since the 1993 outbreak in Milwaukee, US (Fayer, 2008). Ingestion of a small number of Cryptosporidium oocysts of can lead to potentially fatal consequences for immuno-suppressed individuals and it is thought that infection can be caused by even a single oocyst (Rose, 1997). At present, no specific drug treatment exists for cryptosporidiosis. *C. parvum* oocysts are biologically dormant yet resistance to the levels of chlorine routinely used in potable water treatment thus making waterborne transmission of cryptosporidiosis one of the most prominent public health concerns worldwide (Fayer, 2008).

The main source of *C. parvum* oocysts is from infected calves when agricultural water including runoff, infiltration and subsurface flow from dairies, calving house, silage and grazing lands may be loaded with high concentrations of oocysts. Thus, the fate and transport of *Cryptosporidium* oocysts in the soil is of critical importance to manage and assess risk (Pachepsky et al., 2006). Thus, three key processes are evaluated in this study, including i) oocyst survival in the soil, ii) the attachment of oocysts to soil particles and iii) the transport of oocyst with physical straining in porous soils. Our study may provide an insight into the oocyst life cycle in the soil and help us make a better risk assessment.

2. The fate of *Cryptosporidium* oocysts in the soil

Cryptosporidium oocysts can retain infectivity for months and resist environmental stresses more readily than many other pathogens because of a hard protective wall. As a result, characterisation of the fate of oocysts in the soil has received much attention. In general, the survival of oocysts in the soil slows down exponentially with time. To account for shoulder

and tailing effects, a first-order exponential model has usually been used to simulate the die-off curve of oocysts in soils with Eq. 1 (Peng et al., 2008):

$$y_t(t) = y_0 e^{-Kt}, \quad (1)$$

where K (dimensionless) is the die-off rate coefficient, y_0 and y_t are the oocyst number at initial condition and time t (any suitable unit of time), respectively. If normalized by initial oocyst number, Eq. 1 can be rewritten as:

$$y_t'(t) = e^{-Kt} \quad \text{or} \quad \ln y_t'(t) = -Kt, \quad (2)$$

where

$$y_t'(t) = \frac{y_t(t)}{y_0}. \quad (3)$$

In Eq. 2 K is independent of the initial oocyst number and represents a constant die-off rate (K) over the entire incubation period. Alternatively, for a given percentage of inactivated oocysts, K is inversely proportional to the incubation time. Using Eq. 2 it is possible to estimate the infectivity of oocysts at a given time.

2.1 Effect of soil moisture on oocyst survival

Besides temperature, oocyst survival is subjected to stress in soils driven by the interaction of soil moisture and texture. Soil water potential indicates whether oocysts are exposed to wet or dry conditions. Desiccation is probably lethal to oocysts. Walker et al. (2001) reported that decreasing soil water potential by adjusting NaCl solution linearly increased the rate of population degradation. Nasser et al. (2007) also found incubation for 10 days in dry loamy soil at 32^{o}C resulted in a 3-log$_{10}$ reduction in oocyst infectivity, but a saturated soil caused only 1-log$_{10}$ reduction at a similar temperature. Jenkins et al. (2003) reported the estimated K values were increased from 0.014 day^{-1} to 0.416 day^{-1} at 25^{o}C when soil water potential was decreased from -0.10 MPa down to -3.2 MPa, but they also pointed out that when soil water potential indicated a water content in excess of field capacity (> -0.10 MPa), then the effect on oocyst infectivity was negligible. Jenkins et al. (2002) did not find any effect on oocyst inactivation of soil water potential in the range -0.033 to -1.5 MPa using a sentinel chamber. This discrepancy compared to other reports perhaps resulted from the one open end of the sentinel chamber being exposed to free water, through which the water potential of the soil in the chamber could be mediated by the moisture of the surrounding soil. Published results suggest that soil water potential or moisture content can influence oocyst die-off rate.

2.2 Effect of soil texture on oocyst survival

Soil texture, a static property of the soil also appears to affect oocyst survival in the soil. Jenkins et al. (2002) reported oocyst survival appeared to be significantly greater in a silt loam soil (50% silt, 16% clay) than in silty clay loam (69% silt, 29% clay) and loamy sand (14% silt, 5% clay). Davies et al. (2005) found oocysts were inactivated faster in loam soil (27% silt, 24% clay) than in clay loam (55% silt, 38% clay). Soil particles probably do not directly affect oocyst survival, but they can modify oocyst metabolic activity through changing other physiochemical and biological soil properties and their attachment properties.

3. The attachment of *Cryptosporidium* oocysts in the soil

As mentioned, soil particles affected the fate of *Cryptosporidium* oocysts. Oocysts attachment to soil particles during transport may have a significant influence on retention in the soil environment and thus longevity of the organism. Attached oocysts seem to be protected from inactivation by ultraviolet radiation, temperature or desiccation (Morita et al., 2002; Hijnen et al., 2006; Peng et al., 2008), but may be a part of the general food web of the soil. Soil might be regarded as a habitat that can promote survival and provide a source of oocysts especially after spreading of animal manure and slurry. The interaction between oocysts and soil particles can increase the oocysts' effective settling velocity and play an important role in regulating the transport and retention in agricultural catchments (Searcy et al., 2005; Searcy et al., 2006). Application of bovine manure was found to enhance the attachment of oocysts to soil particles and this mechanism was partially reversible if manure was diluted (Kuczynska et al., 2005). Searcy et al. (2005) reported that more oocysts were attached to iron oxide and kaolinite particles than illite particles because the latter are more negative charged, but Walker and Montemagno (1999) found that oocysts were readily attached to Al_2O_3 but not to Fe_2O_3, SiO_2 and hydrophobic substrates. Therefore, whether or not oocysts attach to soil particles may depend on the particles physical and chemical properties. Unfortunately obtaining data on soil mineralogy is difficult as it is rarely collected during wide area surveys. This means it would be very useful to establish whether oocyst attachment can be related to more commonly measured soil properties that can be found in may soil surveys and could therefore be used for improving quantitative environmental risk assessments.

Soil code	Sand§	Silt§	Clay§	SOC¶	CEC¶	Total pore	Macropore¶	K_s¶	pH
	––––– g kg^{-1} –––––				mmol kg^{-1}	–– cm^3 cm^{-3} ––		cm day^{-1}	[-]
S02	445	397	159	27	169	0.48	0.11	2.94×10^{3}	6.08
S01	373	440	187	41	202	0.57	0.06	7.00×10^{2}	5.72
CG	357	431	212	44	187	0.53	0.10	4.42×10^{2}	5.32
EG	355	424	221	48	212	0.60	0.04	9.26×10^{1}	6.57
OG	473	364	162	30	151	0.53	0.04	7.30×10^{1}	5.60
JSG	668	217	116	16.3	92	0.42	0.01	2.16×10^{1}	6.33
KG	386	513	102	91	259	ND#	ND#	$<1.0\times10^{0}$	6.30
RG	394	444	162	38	163	ND#	ND#	$<1.0\times10^{0}$	6.56
CCG	288	496	217	72	225	ND#	ND#	$<1.0\times10^{0}$	5.29

§Sand: 0.05-2 mm, Silt, 0.002-0.05 mm, Clay, <0.002 mm.
¶SOC, soil organic carbon, CEC, Cation exchange capacity, Macropore, >60 µm diameter, K_s, saturated hydraulic conductivity of distilled water.
#ND, not determined.

Table 1. Selected soil properties of nine soils present in permanent pastures in Ireland

In the laboratory, we established an attachment experiment to analyse how soil properties influence the interaction between oocysts and soil particles. Nine typical grassland soils

from Ireland with a limited range of soil texture but a wide range of soil organic C were used (Table 1). The soils were selected by two reasons: (i) those previously identified as being characteristic of Irish grassland agriculture (CG, EG, OG, and RG soils) (Ryan and Fanning, 1996); and (ii) those found in a specific study catchment used for developing risk assessment associated with *Cryptosporidium* transport (S01, S02 and KG soils). In addition, two other soils (JSG and CCG) were selected to increase the range of soil texture in study. For each soil a composite disturbed sample from five points around a a field was taken, air-dried and ground to <2 mm for determining soil properties. Routine methods of the Institute of Soil Science, Chinese Academy of Sciences (Ru, 2000) were used to analyse basic soil properties: particle-size distribution was determined by the pipette method, particle density was measured by pycnometer method, soil organic carbon was by oxidation with potassium dichromate, cation exchange capacity (CEC) by the ammonium acetate method, and pH by a pH meter, saturated hydraulic conductivity (Ks) by a constant head method. Table 1 presents the measured properties of each soil used.

3.1 Oocysts preparation

C. parvum oocysts were purchased from Creative Science Company, UK. They were purified from manure of experimentally infected calves by sucrose and Percoll gradient centrifugation and water washes, and stored in 0.1% phosphate buffered saline solution at 4 ^{o}C before use. The number of oocysts in the stock solution was about 5.0 x 10^{8} oocysts ml^{-1}. Oocysts ranged from 3.9 to 5.9 μm in diameter, and their density was between 1.0 and 1.1 g cm^{-3}.

3.2 The batch attachment experiment

Batch experiments were conducted by spiking ca. 2×10^{4} oocysts into 10 ml, pH 7.0 <2 mm soil particles solution in which particle concentration was 2 mg ml^{-1}. A blank experiment (no particles) was run as a reference. Each treatment was run in triplicate. The tubes were rotated overnight in dark room at 15 ^{o}C to let oocysts attach to particles adequately. The mixed suspension was then stood on a vibration-free bench. After 30 min, the top 1 ml (ca. 6.4 mm height) was aspirated either from an oocyst-particle tube or from a blank treatment for direct oocyst staining. The percentage of particle-attached oocysts was estimated by the ratio of the oocysts concentration from the oocyst-particle solution ($Oocyst_{particle}$) to the oocyst concentration from the blank treatment ($Oocyst_{blank}$) as counted in the top 1 ml.

$$Oocyst_{attached}\% = (1 - \frac{Oocyst_{particle}}{Oocyst_{blank}}) \times 100 \quad (4)$$

3.3 Oocyst staining protocol

The oocyst staining followed the instructions of the Dynabeads® anti-Cryptosporidium kit (Invitrogen, Norway). Briefly: (i) each sample was thoroughly vortexed, a 100 μl aliquot was pipetted into a slide well (diameter 12 mm) and air dried at room temperature; (ii) two drops of methanol were added to each well and allowed to air dry; (iii) 50 μl of a combined fluorescein isothiocyanate (FITC) conjugated anti-Cryptosporidium monoclonal antibody was added to each well; (iv) the slide was placed in a humid chamber at room temperature for 60 min; (v) monoclonal antibody was aspirated from the well and washed twice with PBS; and (vi) the slide was covered with a glass slip. The stained slides were enumerated

immediately at 200× magnification with an epifluorescence microscope (Olympus, Japan) containing a filter cube with an emission of 530 nm and an excitation wavelength of 490 nm.

3.4 Relation between *Cryptosporidium* oocysts and soil particles

The relationship between oocyst and soil particles for the nine test soils (Figure 1) indicated that the KG soil had a poor interaction with oocysts. In EG and RG soils, oocysts showed a strong attachment to soil particles. For the other six test soils the percentage of particle-free oocysts was between 50% and 65%. The attachment of oocysts depended on fine particle content ($r=0.611$, $P<0.1$). Muirhead et al. (2006) reported that pathogenic *E. coli* appeared to attach preferentially to small particles (<2 μm), which is consistent with the result that the percentage of particle-free oocysts was negatively related to clay content, but not to silt or sand content.

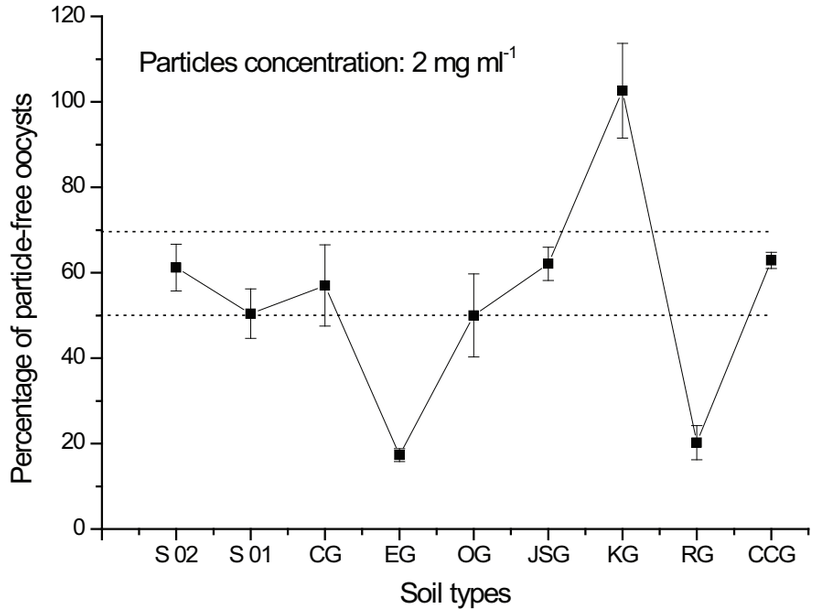

Fig. 1. Oocyst-particle attachment of 9 test soils

Of the 9 test soils, over 35% of oocysts were attached to soil particles with the exception of the high organic carbon KG soil. Negative charged soil organic matter may prevent oocysts, which also have a negative surface charge, from attaching to soil particles. Although there was no significant relationship between attached oocysts and soil organic carbon ($r=-0.492$, $P=0.18$), the sand-free soil organic carbon was observed to be negatively related with attached oocysts ($r=-0.681$, $P<0.05$). This finding is consistent with the results of Searcy et al. (2005) who reported that considerably less oocysts were removed from suspension in the present of more negative charged illite than in the presence of iron oxide or kaolinite. The electrostatic repulsive forces should increase as the suspended particles become more negative. The hydrophobicity of soil organic matter may also contribute to the reduced attachment of oocysts, which is supported by results from Walker and Montemagno (1999) who observed no sorption of oocysts onto hydrophobic substrates.

4. The transport of *Cryptosporidium* oocysts in the soil

The transport and retention behavior of Cryptosporidium oocysts has been examined using homogeneous columns under steady-state, constant flux (CF) conditions controlled by a peristaltic pump (Brush et al., 1999; Harter et al., 2000; Logan, et al., 2001; Bradford and Bettahar, 2005; Hijnen et al., 2005). These studies concentrated on how oocysts moved through homogeneous porous media as affected by the particle size, flux rate or solution chemical properties. Decreasing the median sand size tends to produce lower effluent concentrations and greater oocyst retention near the column inlet (Harter et al., 2000; Logan et al., 2001). High flux rate can produce more oocysts in effluents than low flux rate (Harter et al., 2000; Hijnen, et al., 2005). Hus et al. (2001) and Tufenkji et al. (2005) found that the oocyst removal efficiency depended on ionic strength and solution pH. For the colloid-sized oocysts breakthrough curve (BTC), three physical mechanisms of attachment, detachment and irreversible straining may be involved in the tailing and heterogeneous distribution of oocysts at depth (Bradford et al., 2003; Bradford and Bettahar, 2005). However, the pump used in some of these experiments may force oocysts to pass through smaller pores in order to maintain the CF conditions. In other words, under CF conditions, the pressure applied by the pump will increase with clogging in order to keep a CF. The transport behaviour and breakthrough curve may be different from those under the constant pressure conditions (CP). For the same initial flow rate, we hypothesized that the CF condition may therefore overestimate the oocyst transport potential compared with CP conditions.

In this study, we tested three different porous materials. One coarse sand, one fine sand and soil material <2 mm sieved from a Haplic Cambisol soil from CG site, were uniformly repacked into stainless steel cylinders (20 cm in height, 10 cm in diameter). The median grain size (d_{50}) of coarse sand, fine sand and disturbed soil was 0.12, 0.17 and 0.32 mm, respectively (Table 2). The uniformity index (d_{60}/d_{10}) was 2.4 for coarse sand, 2.3 for fine sand, and 8.2 for disturbed soil. The three homogeneous columns were used to compare oocysts BTC between constant flux (CF) and constant pressure head (CP) systems. For each porous medium, the initial volumetric flow rate between the CF and CP systems was identical.

Porous media	d_{10} (mm)†	d_{50} (mm)	d_{60} (mm)	Uniformity Index (d_{60}/d_{10})	Porosity ($cm^3\ cm^{-3}$)	Ks§ ($cm\ min^{-1}$)
Coarse sand	0.07	0.15	0.17	2.4	0.44	1.7
Fine sand	0.06	0.12	0.14	2.3	0.43	1.3
Disturbed soil	0.05	0.32	0.41	8.2	0.59	0.13

†d_{10}, d_{50}, d_{60} mean 10%, 50%, 60% of the mass finer than the size, respectively
§K_s, saturated hydraulic conductivity

Table 2. Properties of the three homogeneous porous media studied

4.1 The oocysts transport experiment

Prior to BTC investigation, the columns were saturated from the bottom with distilled water for two days. Then about 1-2 pore volume of 0.1 M NaBr solution ran through the column from top to bottom, followed by another 1-2 pore volume of 2.0×10^3 oocysts ml^{-1} solution.

The oocysts reservoir was placed on a stirrer to keep the oocyst homogeneously mixed during the experiment. Br^- and oocysts transport was pushed through the column under a 22 cm CP between the inlet and the outlet or under the CF conditions controlled by a peristaltic pump (Figure 2). Each porous medium was run in triplicate.

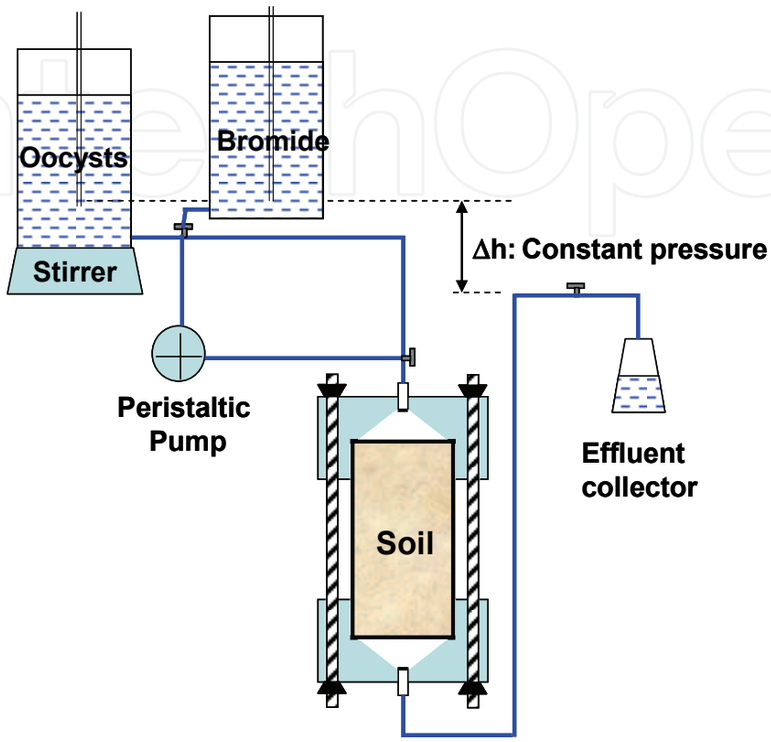

Fig. 2. A schematic diagram of the soil column experiment setup employed in the Br- and oocysts breakthrough study under a constant pressure (CP, Δh=22 cm in this study) or under a constant flux (CF) controlled by a peristaltic pump

4.2 Simulation analysis

The transport of colloid-sized *Cryptosporidium* oocysts can be described by the convective-dispersion equation (CDE), which has been widely used to model solute transport (Jury, 1991). To describe oocysts transport through saturated columns, we employed the one-dimensional CDE:

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \mu C \tag{5}$$

where C is the reduced concentration of the liquid phase (dimensionless), t is time (min), x is vertical distance in the direction of flow (cm), D is the hydrodynamic dispersion coefficient ($cm^2\ min^{-1}$), v is the average water velocity ($cm\ min^{-1}$), and μ is the first-order rate decay coefficient describing straining/filtration (min^{-1}). R is the retardation factor, defined as

$$R = 1 + \frac{\rho K_d}{\theta} \tag{6}$$

where ρ is the bulk density of the porous media (g cm^{-3}), θ is the volumetric water content, and K_d is the distribution coefficient describing solute adsorption to the solid phase (cm^3 g^{-1}). Using the computer program CXTFIT for solving nonlinear CDE regression (Toride et al.,1995), we firstly obtained the fitted *v* and *D* parameters from the tracer Br^- BTC, with *R* = 1.0 and μ =0 min^{-1}. Pore water *v* and *D* of the oocyst BTC were assumed to equal those of the Br^- trace in each column. With this assumption, we estimated *R* and μ of the *C. parvum* oocyst BTC.

4.3 Transport of oocysts in the columns

The relative concentration (C_i/C_0) of Br^- and oocysts in effluents as a function of pore volume following step input (Figure 3) reduced the experimental time and the time-consuming enumeration, as compared to using a pulse step method, because the latter requires definition of the rising and falling limbs. For colloid-sized *C. parvum* oocysts transport, three physical mechanisms of straining, attachment and detachment have recently been discussed using results from the pulse step method (Harter et al., 2000; Bradford et al., 2004; Bradford and Bettahar, 2005). Straining was identified as perhaps the most important mechanism. Use of a step input method may highlight the straining mechanism, but any detachment was ignored. Therefore, the retardation parameter *R* and the straining factor μ of oocyst BTC were estimated from CDE theory (Table 3).

Porous media	Flux system	Bromide			Oocysts		
		v	*D*	r^2	*R*	m	r^2
Coarse	CF	3.60±0.13 a†	1.62±0.39 a	1.00±0.00	0.94±0.04 a	0.00±0.00 a	0.91±0.03
sand	CP	3.90±0.12 a	1.76±0.61 a	0.99±0.01	0.79±0.09 a	0.00±0.00 a	0.90±0.05
Fine	CF	3.10±0.04 a	0.89±0.11 b	1.00±0.00	0.92±0.07a	0.01±0.00 b	0.96±0.01
sand	CP	3.10±0.29 a	1.10±0.04 a	1.00±0.00	0.89±0.14 a	0.07±0.01 a	0.96±0.02
Disturbed	CF	0.81±0.04 a	4.30±0.97 a	0.98±0.00	1.40±0.57 a	0.13±0.02 a	0.36±0.19
soil	CP	0.76±0.21 a	12.47±5.4 a	0.98±0.02	5.98±6.25 a	0.21±0.11 a	0.48±0.28

†Different letters indicate significant difference between CF and CP systems at P<0.05

Table 3. Fitted parameters of the convective-dispersion equation using the CXTFIT package for the homogeneous porous media. *v* = pore water velocity (cm min^{-1}), *D* = hydrodynamic dispersion coefficient (cm^2 min^{-1}), *R* = retardation factor, μ = straining factor (min^{-1}), and r^2 = squared relation coefficient. CF = constant flux, CP = constant pressure. Mean±standard deviation (n=3)

For the coarse sand column, the colloid-sized oocyst BTC was earlier than the tracer Br^- in both CF and CP systems (Figure 3). This phenomenon of oocysts travelling faster than the tracer Br^- was also observed in fine sand column under the CF conditions. However, a remarkable delay took place in the disturbed soil material. The results were consistent with the fitted retardation factor *R* values less than 1 for the coarse and fine sand column but

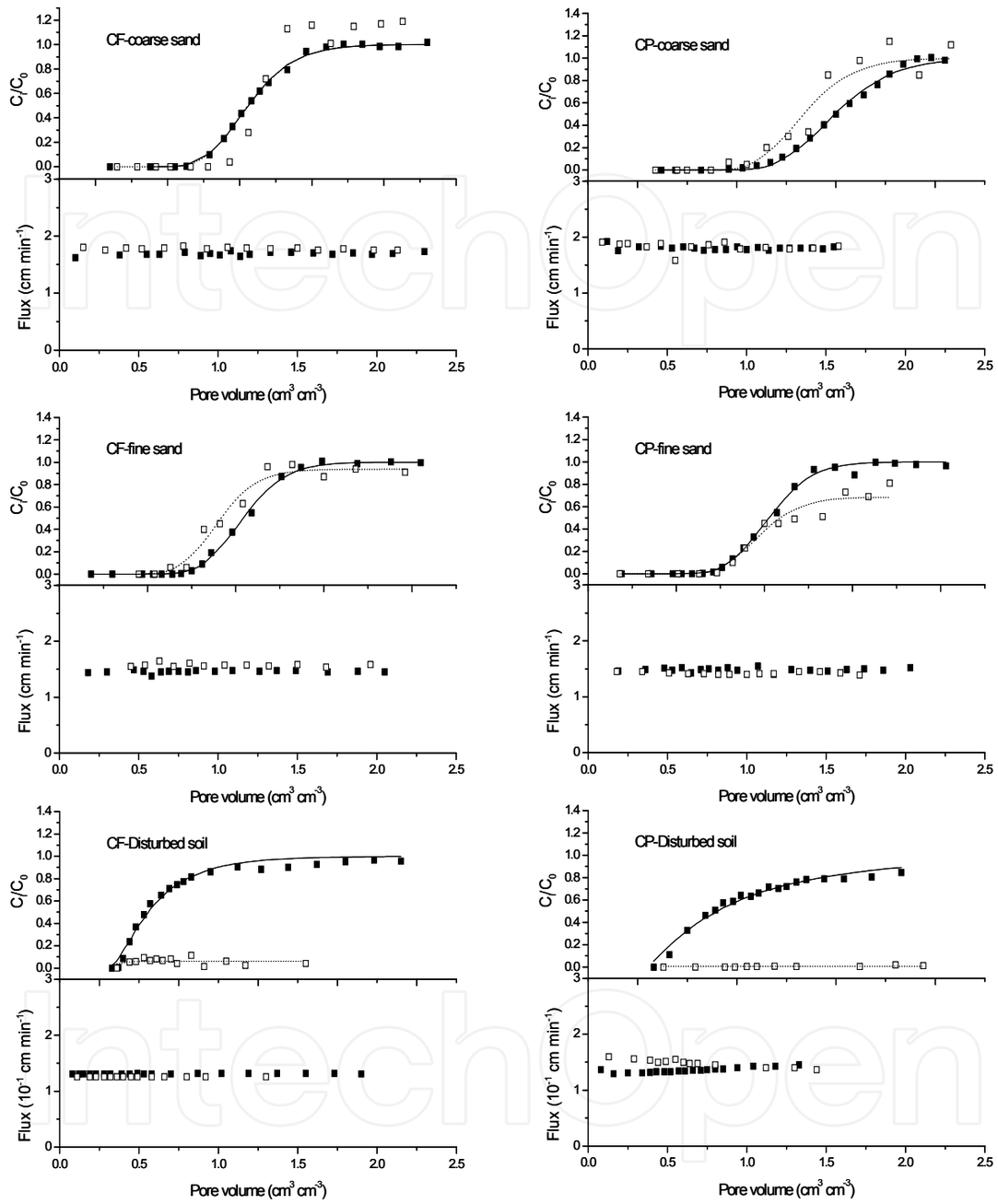

Fig. 3. Comparison of the breakthrough curves of Br^- (solid squares) and oocysts (open squares) and their water flux as a function of pore volume between CF and CP systems for the three homogeneous pore media. The lines of Br^- (solid) and oocysts (dot) are the curves fitted with Equation 1. C_0 is the applied concentration, C_i is the measured concentration

larger than 1 for the disturbed soil (Table 3). The *R* values less than 1 in this study agree with previous reports (Brush et al., 1999; Harter et al., 2000) that oocysts were excluded from the pores finer than their size and from the margins of the pore throats, but this was not true

for tracer Br^-. The size exclusion and pore inaccessibility would most likely increase the average pore velocity of colloid-sized oocysts.

Both CP and CF systems displayed an identical steady-state water flux either in ionic Br^- transport or in colloid-sized oocyst transport in the coarse sand column. From the simulation results, there was no significantly difference of the means of the parameters v, D, R and μ. In the coarse sand column, $\mu=0$ indicated that physical straining was negligible. In the fine sand column, oocyst BTC was consistent with the Br^- BTC under the CF conditions, but slower at the later part under the CP conditions, although the water flux remained at a steady state in both Br^- and oocyst transport. The parameters of D and μ were significantly greater under the CP conditions than under the CF conditions, which further demonstrated that more oocysts were retained in the former, even when the water flow rates were identical. The oocysts BTC was overestimated when the consequences of physical straining were overlooked in the CF system. The small values of μ, 0.01 and 0.07 min^{-1} in the CF and CP systems, respectively, showed that the straining of oocysts was not predominant in the fine sand column. The physical straining that occurred in the fine sand meant a steady state of water flow was maintained, but with sufficiently reduce effluent oocyst concentrations. The grain size-dependent oocyst transport was in accordance with other findings (Harter et al., 2000; Bradford et al., 2003; Bradford et al., 2005), which indicate that decreasing grain size tends to produce lower effluent concentration and greater oocyst retention in the column.

In the disturbed soil column, oocyst BTC was much slower than Br^- in both CF and CP systems. Oocysts passing through columns were much less under the CP conditions than under the CF conditions. Under the CP conditions, the water flux during the oocyst breakthrough process always decreased over the entire experiment, whereas the water flux during the Br^- transport increased due to some fine soil particles leaching from the column. The decrease in the water flux resulted from physical straining and trapping of oocysts in the soil pores (Bradford and Bettahar, 2005), or from change of ionic strength (Hsu et al., 2001; Tufenkji et al., 2004). The fitted D, R and μ values in the CP system were greater than in the CF system, although the simulation was poor for the oocyst BTC ($r^2 < 0.5$).

Oocyst concentration decreased exponentially from the inlet to the outlet in coarse and fine sand columns (Figure 4), which is in generally agreement with the shape of the oocyst spatial distribution observed by Bradford and Bettahar (2005) and Harter et al. (2000). Due to a very low recovery for soils, the measurement was not applied to the disturbed soil samples. In coarse sand, a similar mass fraction in effluent (51.5-56.6%) was observed under the CF and the CP conditions. This identical result was for the fraction retained in the columns (29.4-29.9%). In fine sand, however, a significantly greater mass fraction in the effluent was under the CF conditions compared to the CP conditions, although the oocyst fraction retained in the columns was similar.

From the results of the three homogenous columns, the physical straining was more pronounced in disturbed soil than in the sand columns. This may be caused by oocysts preferentially attached to soil particles and by finer pore size distributions in the disturbed soil columns. The consequences of physical straining of oocysts through fine sand were not observed under the CF conditions. Use of a peristaltic pump to keep the flux at a steady state has been widely applied to investigate colloid-sized oocysts transport (Brush et al., 1999; Harter et al., 2000; Tufenkji et al., 2004; Bradford and Bettahar, 2005; Hijnen et al., 2005; Bradford et al., 2006). However, the pump may force oocysts to pass through pores or pore necks which are smaller than their critical size. As a result, the oocyst BTC may be

overestimated in the CF system when physical straining takes place. On the other hand, if pore space is large enough for oocyst transport, the physical straining may become a minor mechanism, as occurred in the coarse sand column even in the CP system.

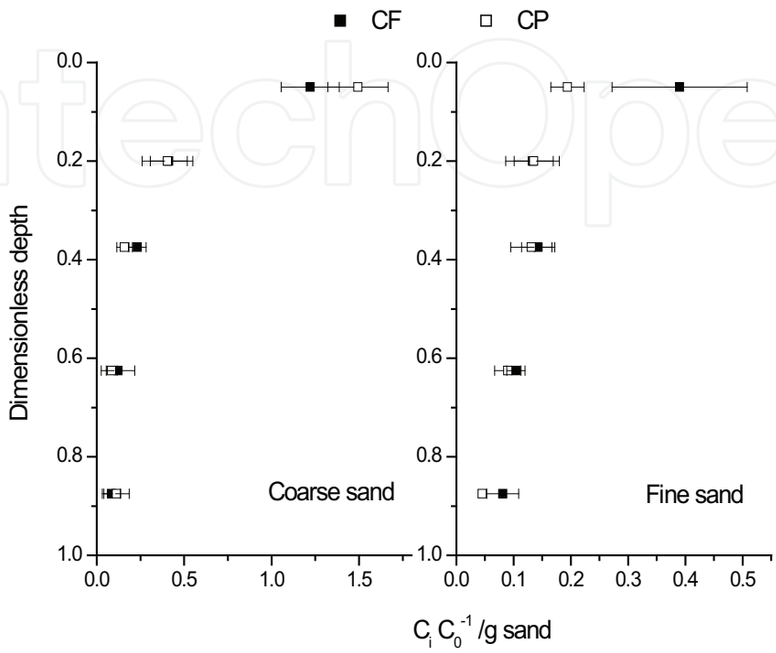

Fig. 4. Comparison of oocysts retention profiles in the coarse and fine sand columns between CF and CP systems. C_0 is the applied concentration, C_i is the measured concentration

Porous media	Flux system	$Mass_{effluent}$	$Mass_{retained}$	Total recovery (%)
Coarse sand	CF	56.6±1.0a	29.4±7.0a	86.6±6.0
	CP	51.5±8.2a	29.9±3.4a	81.4±11.2
Fine sand	CF	46.7±4.9a	14.1±3.3a	60.8±6.9
	CP	30.7±1.1b	10.6±0.7a	41.4±1.8
Disturbed soil	CF	6.8±2.3a	ND	ND
	CP	0.4±0.2b	ND	ND

†Different letters indicate significant difference between CF and CP systems at $P<0.05$
ND=Not determine

Table 4. Oocyst fractions in effluents and retained in the columns. CF = constant flux, CP = constant pressure. Mean±standard deviation (n=3)

5. Conclusions

For colloid-sized *Cryptosporidium* oocysts the fate and transport processes depend much on the soil physical and chemical properties. The survival of oocysts is affected by soil temperature, moisture and texture. Oocysts preferentially attach to fine particles, but soil organic carbon prevents some oocyst attachment to soil particles. The transport of oocysts under constant pressure head conditions displays a decrease of water flux and a reduction of oocysts concentration in effluents as a result of physical straining, which is perhaps underestimated under constant flux conditions by a peristaltic pump. The understanding of the fate and transport of oocysts in the soil is a basic knowledge requirement in order to properly assess risk associated with *Cryptosporidium* transport through the wider environment.

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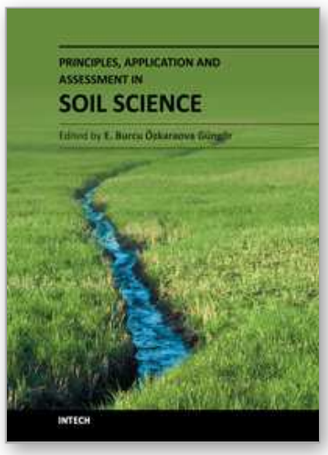

Principles, Application and Assessment in Soil Science
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Our dependence on soil, and our curiosity about it, is leading to the investigation of changes within soil processes. Furthermore, the diversity and dynamics of soil are enabling new discoveries and insights, which help us to understand the variations in soil processes. Consequently, this permits us to take the necessary measures for soil protection, thus promoting soil health. This book aims to provide an up-to-date account of the current state of knowledge in recent practices and assessments in soil science. Moreover, it presents a comprehensive evaluation of the effect of residue/waste application on soil properties and, further, on the mechanism of plant adaptation and plant growth. Interesting examples of simulation using various models dealing with carbon sequestration, ecosystem respiration, and soil landscape, etc. are demonstrated. The book also includes chapters on the analysis of areal data and geostatistics using different assessment methods. More recent developments in analytical techniques used to obtain answers to the various physical mechanisms, chemical, and biological processes in soil are also present.

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